BATCH ETHANOL FERMENTATION USING GLUCOSE DERIVED FROM TAPIOCA FLOUR STARCH BY *Saccharomyces Cerevisiae FOR* EFFECT OF TEMPERATURE AND AGITATION RATE

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"I hereby declare that I have read this thesis and in
my opinion this thesis is sufficient in terms of scope and
quality for the award of the degree of Bachelor of Chemical Engineering
(Biotechnology) "

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Date : May 2008

DECLARATION

I declare that this thesis entitled "Batch Ethanol Fermentation Using Glucose Derived from Tapioca Flour Starch by *Saccharomyces cerevisiae* for Effect of Temperature and Agitation Rate" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature : ……………………………………….
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Date : May , 2008

DEDICATION

Special Dedication to my Mother and Father,
My family members that always love me,
My friends, my fellow colleague, Kak Zai & Abang Solihon
and all faculty members.

For all your Care, Support and Believe in me.

Sincerely
Mohd Azimie Bin Ahmad

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ABSTRACT

Bioethanol can be produced from amylose and amylopectin that originate from various sources of biomass. This study was focusing on the production of ethanol from glucose derived from tapioca flour starch using *Saccharomyces cerevisiae*. The objectives of this study are to determine the yield of ethanol produced from certain amount of tapioca flour, the effects of the fermentation temperature and agitation speed in fermentation process. Enzymatic process is purposely done to convert the tapioca flour starch to its glucose. Two types of enzyme were employed for these processes which were α-amylase and amyloglucosidase. This study is done in batch mode of aerobic fermentation process. The temperature and agitation used in this experiment are 30, 35, 37°C and 100, 200, and 300 rpm. Determination of cell growth concentration in inoculation process plays an important role to ensure the microbial activity and determination of microbe growth. Results show that yield of ethanol production will increase as the temperature and agitation increase until it reaches the optimum point. From this study, it was observed that optimum condition for ethanol fermentation by *Saccharomyces cerevisiae* was at 35°C with agitation speed of 200 rpm

ABSTRAK

Bioetanol boleh dihasilkan daripada amilose dan amilopektin yang boleh diperolehi dari pelbagai sumber biojisim. Kajian ini dijalankan untuk menghasilkan bioethanol daripada glukosa yang diperolehi daripada kanji tepung ubi kayu dengan menggunakan mikroorganisma dikenali sebagai *Saccharomyces cerevisiae*. Objektif kajian ini adalah untuk menentukan kadar penghasilan etanol dari kuantiti tertentu tepung ubi kayu yang digunakan, juga mengkaji kesan fermentasi etanol akibat perubahan suhu dan perubahan halaju adukan. Proses penukaran kanji tepung ubi kayu kepada glukosa melibatkan penggunaan dua jenis enzim iaitu α-amylase dan amyloglucosidase. Fermentasi etanol dijalankan dalam mod sekumpul secara aerobik. Perubahan suhu yang dikaji adalah pada 30°C, 35°C dan 37°C dan kadar halaju pengadukan pula adalah 100, 200 dan 300 rpm. Keputusan eksperimen menunjukkan penghasilan etanol akan meningkat dengan peningkatan suhu fermentasi dan kadar kelajuan pengadukan. Daripada kajian ini, keadaan optimum untuk fermentasi etanol oleh *Saccharomyces cerevisiae* adalah pada 35°C dengan kelajuan adukan pada 200 rpm.

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%	-	percent
°C	-	°Celcius
μg/ml	-	microgram per mililiter
g	-	gram
g/ml	-	gram per mililiter
kg	-	kilogram
L	-	liter
L/h	-	liter per hour
ml	-	mililiter
mm	-	milimeter
rpm	-	rotation per minute
v/v	-	volume per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fermentation may be thought of as chemical reactions catalyzed by living cell. Fermentation is the anaerobic or aerobic conversion of sugar to carbon dioxide and alcohol by microorganisms. Variety of products can be produced by fermentation, such as pharmaceuticals, organic acids and alcohols. However, to be commercially viable, the bioprocesses must be economically competitive with alternative processes, such as petrochemical manufacturing. Advances in recombinant-DNA technology allow fermentation to mass produce chiral and complex biomolecules (such as human insulin) more economically than by other means. Most widely known fermentation product is ethanol. Batch yeast fermentations have been used for hundreds of years to produce alcoholic beverages. Ethanol can be produced from a variety of plant-derived raw materials, including agricultural wastes. Currently, there is a small market for ethanol as a fuel additive, but for ethanol to successfully compete with petroleum, additional improvements in the process economics are needed. The use of baker's yeast, such as *Saccharomyces cerevisae*, that grow faster and give higher ethanol yields than the conventionally used yeast could provide such an improvement. Objectives of this study are to carry out an ethanol fermentation using *S. cerevisiae* utilizing cheap carbon sources and to study optimum condition (agitation speed and temperature) of the fermentation.

1.2 Problem Statement

S. cerevisiae is facultative anaerobic yeast which ferments hexose sugars under aerobic and anaerobic conditions (Hohmann, 1997). Yeast will be grown in glucose-rich medium where it will utilize the sugar at different modes to produce energy depending upon the specific growth conditions.

Organisms employ cellular respiration to harvest energy in the form of ATP in the presence of oxygen (Campbell, 1996). Enzymatic activity is the driving force behind the three main stages of cellular respiration – glycolysis, the Krebs cycle and the electron transport chain. Without oxygen, organisms undergo fermentation which only involves the first stage of cellular respiration, glycolysis, and subsequently requires less enzymes (Campbell, 1996). Cellular respiration yields 38 ATP molecules while fermentation only produces 2 ATP molecules (Campbell, 1996). Organisms rely on ATP to drive their cellular processes and promote growth. Very little growth occurs during lag phase where the yeast acclimate themselves to their environment and synthesize enzymes necessary to catabolize nutrients (Hohmann, 1997). The yeast will synthesize fewer enzymes in anaerobic environment than in an aerobic environment since fermentation requires fewer enzymes than cellular respiration (Mills, 1967). It is possible that yeast growth will be inhibited under anaerobic conditions since fermentation produces alcohol, which also decreases pH over time and kill the yeast (Miller, 1900). Consequently, the partial pressure of oxygen is a key factor affecting yeast growth in addition to temperature and pH. The optimum temperature growth range of yeast is between 25°C and 37°C (Miller 1900). The optimum agitation speed range of yeast is between 100 to 200 rpm (Kotter, 1993).

1.3 Research Objectives

1) To determine ethanol yield from fermentation of *Saccharomyces cerevisiae* utilizing starch from tapioca flour.
2) To study the effect of different fermentation temperature and agitation speed on ethanol fermentation in flask.

1.4 Research Scope

~~1)~~ Glucose conversion from starch derived from tapioca flour by enzymatic hydrolysis and two types of enzyme was employed for these processes which were α-amylase and amyloglucosidase.

2) Variation of process condition for temperature 30°C, 35°C, 37°C and for agitation speed is 100 rpm, 200 rpm, and 3000 rpm and its effect on bioethanol production in fermentation process.

1.5 Research Benefits

1) Provide an optimum operation condition (temperature & agitation speed) for continuous ethanol production.
2) Ethanol fermentation using cheap carbon source (sugar derived from tapioca flour starch).

CHAPTER 2

LITERATURE REVIEW

2.1 Bioethanol

Ethanol or ethyl alcohol (CH_3CH_2OH) is an important organic chemical because of its unique properties, and therefore can be used widely for various purposes. Under ordinary conditions, ethanol is a volatile, flammable, clear, colorless liquid, miscible in both water and non-polar solvents.

The production of ethanol has two routes: synthetic and biological. The synthetic ethanol production is commonly carried out by a catalytic hydration of ethylene in vapor phase and often as a by-product of certain industrial operations (Logsdon, 2006). Ethanol produced from this process is mostly used as a solvent (60%) and chemical intermediate (40%). Fermentation ethanol production accounts for 93% of the total ethanol production in the world. The ethanol is produced from fermentation of sugar extracted mostly from crops. *S. cerevisiae* is most popular microorganism used for ethanol production due to its high ethanol yield and high tolerance to rather high ethanol concentration. Ethanol is mostly used as fuels (92%), industrial solvents and chemicals (4%) and beverages (4%) (Logsdon, 2006).

Crops were main feedstock used for ethanol fermentation nowadays, Brazil is the largest ethanol producer and uses sugar cane as feedstock, while the USA in second place by using corn as feedstock (Rosillo-Calle and Walter, 2006). However these crops are also food for human and animals, thus the expansion of production capacity, especially as ethanol becomes a worldwide alternative fuel, is limited by supply of the feedstock. In contrast, cheap starch materials are available as alternative feedstock for ethanol production. Therefore, the use of starch materials for ethanol production is very promising.

In this study, ethanol as fuel and starch derived from tapioca flour as feedstock for ethanol production is in focus. The first part discusses the advantages of ethanol as fuel in regard to combustion as well as to its environmental impact, while the second part discusses the structure of starch materials, followed by the enzymatic hydrolysis process which breaks down of starch to obtain fermentable sugars (glucose).

2.1.1 Ethanol as Fuel

The use of ethanol as fuel goes back to origin of the use of vehicles itself. For example, Henry Ford's Model T. built in 1908, ran on ethanol. It was continued until the availability of cheap petrol effectively killed off ethanol as a major transport fuel in the early part of the 20th century. The energy crisis of the 1970s renewed interest in ethanol production for fuels and chemicals (Marrs, 1975). Although the interest study in the following decade due to oil price, the environmental issue of reducing greenhouse gas, rising vehicle fuel demand, and the security of energy supply sustain the development of ethanol production from renewable resources.

Ethanol is used in vehicle either as a sole fuel or blended with gasoline. As an oxygenated compound, ethanol provides additional oxygen in combustion, and hence obtains better combustion efficiency. The physicochemical properties of some oxygenated high-octane additives are shown in Table 2.1. Since the completeness of combustion is increased by the present of oxygenated fuels, the emission of carbon monoxide is reduced by 32.5% while the emission of hydrocarbon is decreased by 14.5% (Rasskazchikova *et al*., 2004). In addition, the emission of nitrogen oxides is reduced by using ethanol as additive.

Table 2.1: The physicochemical properties of some oxygenated high-octane additives to gasoline. Source: (Rasskazchikova *et al*., 2004)

Properties	Gasoline	Oxygenates		
		Methanol	Ethanol	MTBE
Density at 15.56, kg/m^3	719-779	794	792	742
Heat, kJ/kg Combustion (lower) Evaporation	 41,800- 44200 ~349	 19,934 1104	 26,749 839	 35,123 326
Flash point, °C	-42.8	6.5	12	-28
Octane number Research (RON) Motor (MON)	 90-100 81-90	 107 92	 108 92	 116 101
Reid vapor pressure, kPa	55-103	32	16	54

Methyl-tertiary-butyl-ether (MTBE) has properties similar to those of gasoline, but a higher octane number, and therefore is very suitable for high-octane additive. However, MTBE is reported responsible for groundwater pollution as a result, for example is the leaking of the underground tanks. Low levels of MTBE can make drinking-water supplies undrinkable due to its offensive taste and odor. MTBE has higher water solubility compare with other gasoline constituents, thus is rather difficult to purge from ground water (Rong, 2001). Moreover, biodegradation of MTBE needs lots of oxygen which is almost impossible to carry out naturally in ground water. The use of methanol as oxygenated is limited, or in many countries prohibited, due to its high toxicity, volatility and hygroscopic behavior. Ethanol has become more competitive as an oxygenated fuel especially because ethanol is produced from renewable resources by fermentation, resulting in less dependency on fossil fuel. Moreover, ethanol is less hygroscopic, contains a reasonable heat of combustion, has lower evaporation heat and, most importantly, is not toxic like methanol. In addition, acetaldehyde as a product of partial oxidation of ethanol in the exhaust gas of vehicles is much less toxic than formaldehyde, which is formed when using methanol.

As a high-octane additive, ethanol has drawbacks: emitting acetaldehyde of 2-4 times as much as does gasoline that highly corrosive, which is a function of water content which can bring a negative effect on rubber and plastic, and the blend with gasoline tends to separate in the presence of traces of water (Rasskazchikova *et al.,* 2004). Fortunately, these drawbacks have been overcome. An additional 5% of water in a blend of ethanol and gasoline can reduce the emission of acetaldehyde. Stabilizers like higher alcohols, aromatic amine, ethers or ketones are useful to prevent separation. For example, 2.5-3% of isobutanol stabilized the gasoline-ethanol blend in the presence of 5% water at low temperature of -20°C. Some corrosion inhibitor such as hydroxyethylated alkylphenols and alkyl imidazolines can attain essential anticorrosion resistance. Additionally, polymer industries have developed special material that are resistant to penetration of alcohols (Rasskazchikova *et al*., 2004).

2.1.2 Environmental Impact

The main environmental advantages of fuel ethanol are its sustainability in using a renewable resource as a feedstock, thus promoting independence of fossil fuel, and maintaining the level of greenhouse gas (CO_2). While crops are useful as energy sources for human and animals, some crops like starch or oil-containing crops can be converted to fuels or chemicals. Combustion of these fuels produces CO_2 gas which would be assimilated again by plants. How effectively ethanol reduces greenhouse gas emission has been widely discussed. The issues are mainly related to the net energy content in ethanol, and depend on the assumption of ethanol production routes. A number of life-cycle assessments have been studied, and show that a change from fossil fuel to biofuels could reduce CO_2 emission by factor of 1/2 to 1/5, depending on how significant the use of renewable fuels is at all stages in the process (Bernesson *et al*., 2006; Hu *et al*., 2004; Kadam, 2002; Kim and Dale, 2005; Rosillo-Calle and Walter, 2006; von Blottnitz and Curran, 2006). Ethanol is harmless to the environment. In ground water and soil mixtures, ethanol can be rapidly degraded both aerobically (100 ml/L in 7 days) and anaerobically (100 mg/L in 3-25 days) (Armstrong, 1999). Ethanol in surface water is also rapidly degraded and thus not harmful as long as it is not present in concentrations directly toxic to microorganisms. The half-time of ethanol in surface water is 6.5 to 26 hours. While ethanol releases volatile organic compounds (VOC) due to its low vapor pressure, degradation of ethanol in the atmosphere is also predicted to be rapid.

Exposure of humans to ethanol is harmless. The exposure may be carried out mostly by inhalation of ethanol vapor as VOC, and by body contact or, rarely, ingestion from either blended fuel or denatured fuel. Biological exposures and responses to ethanol are typically evaluated in terms of blood ethanol concentrations (BEC). The endogenous level is 0.02-0.15 mg/dL while the legal limit for vehicle drivers is 80- 100 mg/dL (Armstrong, 1999). In addition, (von Blottnitz and Curran, 2006) studied the potential health effects of gasoline and ethanol engine exhaust fumes. He concluded that the acute toxicity of the exhaust gas of a gasoline-fueled engine is significantly higher than that of an ethanol-fueled engine.

2.2 Structure of Starch Materials

Starch is a complex carbohydrate which is soluble in water. It is used by plants as a way to store excess glucose and can be used as a thickening agent when dissolved and heated. The word is derived from Middle English *sterchen*, meaning to stiffen. The formula for starch is $C_6H_{10}O_5$ (Hedley, 2002). In terms of human nutrition, starch is by far the most important of the polysaccharides. It constitutes more than half the carbohydrates even in affluent diets, and much more in poorer diets. It is supplied by traditional staple foods such as cereals, roots and tubers. Starch contains a mixture of two molecules: amylose and amylopectin. Usually these are found in a ratio with amylopectin found in larger amounts than amylose. Starch is often found in the fruit, seeds, rhizomes or tubers of plants. The major resources for starch production and consumption worldwide are rice, wheat, corn, and potatoes.

2.2.1 Composition and Structure of Starch

Starches are found in a large number of plants as the major carbohydrates reserve and provide an essential source of energy to us. The largest and most important source of starch comes from corn (maize). The other common source of starch comes from wheat, potatoes, tapioca and rice (Marrs, 1975).

Starches have wide commercial use and it extends beyond the food and drinks industries due to its inexpensive and abundant supplies. Starches are used as thickening agents in baby food formulation and semi-solids food such as sauces, custards and pie fillings. They are also used as binding agents in products such as sausages and processed meat. For non food purpose, starches are used mainly in paper, packaging and textiles industries. They are also used as fillers in the pharmaceutical industry for pill manufacture (Marrs, 1975; Galliard, 1987).

Starch are predominantly composed of two polysaccharides macromolecules, amylose (20-30%) and amylopectin (70-80%), which are packed in a form of partially crystalline granules (Barsby *et al*, 2003). Starch is produced as granules in most plants cells and is referred to native when in this particular granular state. Native starches from different botanical sources vary widely in structure and composition, but all granules consist of two major molecular components, amylose and amylopectin, both of which are polymers of α-D-glucose units in the $^4C_1$ conformation. Molecule structure of amylose shown in Figure 2.1, these are linked (1 → 4), with the ring oxygen atoms all on the same side, whereas in amylopectin about one residue in every twenty is also linked (1 → 6) forming branch-points as shown in FIGURE 2.2.

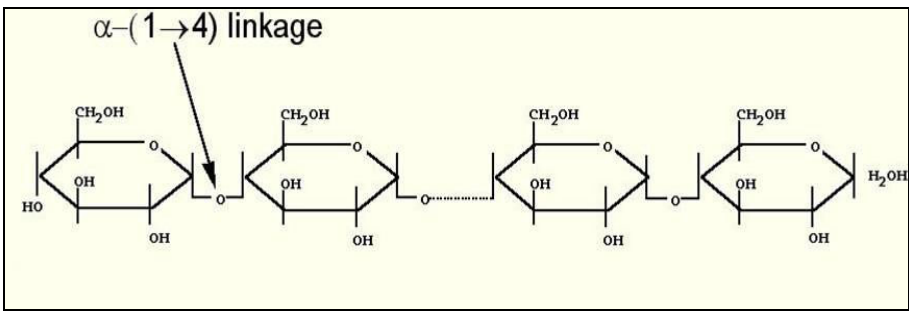

Figure 2.1 *Amylose* molecule structure

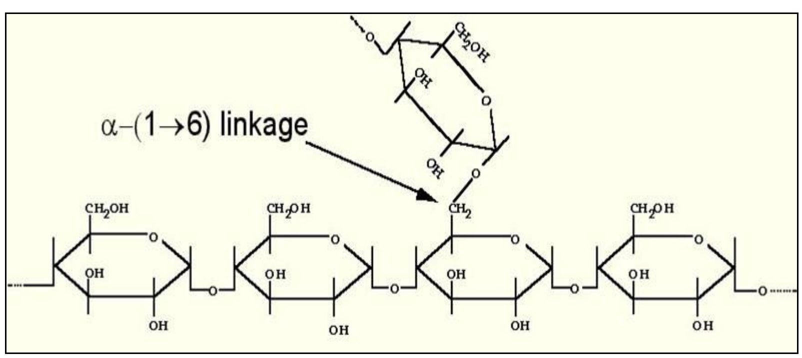

Figure 2.2 Amylopectin molecule structure (Hedley, 2002)